APPENDIX B

[Vector Encoding Papillomavirus Fusion Polypeptide]

TITLE OF THE INVENTION

Vector for Activating the Immune System Against Cells Infected with Papillomaviruses, Papillomaviruses or Fragments Thereof

CROSS REFERENCE TO RELATED APPLICATIONS

This application was filed under 35 U.S.C. § 371, which was the National Stage of International Application No. PCT/DE97/01629, filed 07/30/97.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an adeno-associated virus (AAV) vector comprising a nucleotide sequence that encodes a fusion polypeptide comprising a structural papillomavirus polypeptide and an early papillomavirus polypeptide or fragments thereof, respectively. This vector may be used to activate[suited for activating] the immune system against papillomavirus particles or fragments thereof, and/or cells infected with papillomaviruses, particularly tumor cells [associated to papilloma viruses and sequences thereof, respectively] transformed by papilloma virus infection. This vector may be used as a therapeutic treatment or as a part of a vaccine protocol. [a vaccination agent which contains such a vector and the use of both]

2. Description of Related Art

Papilloma[ ]viruses (PVs) infect the epitheli[um]al cells of a wide range of [man and] animals, including humans. Human papillomaviruses (HPVs) are the cause of benign and malignant growths. HPVs have been found for example in benign[ant, e.g.] warts, condylomas in the genital region, and in malignant[, e.g.] carcinomas of the skin and uterus[,] (e.g., epithelial neoplasms). HPVs may also be responsible[are also considered] for the development of malignant tumors in[of] the respiratory system including squamous cell carcinomas. [In addition, HPVs are considered to be at least jointly responsible for the development of squamous carcinomas of the lungs.]

Papilloma[ ]viruses have an icosahedral capsid that surrounds[without coat which has] a circular double-stranded DNA molecule of about 7900 base pairs. The capsid comprises a major capsid protein (L1) and a minor, capsid protein (L2) but lacks an envelope. The former capsid protein is coded by[the] open reading frame L1 (L1-ORF) and the latter is coded by open reading frame L2 (L2-ORF). *In vitro* expression of L1 or L1 and L2 results[*in vitro*] in the formation of virus-like particles (VLPs). Further, the ability of PVs to transform infected or host cells [The transformation ability of papilloma viruses] is ascribed to the proteins E6 and E7 which are coded by E6- and E7-ORFs, [They are coded by E6 ORF and E7 ORF,]respectively.

Many attempts have been made to stimulate the immune system over cells already infected with PVs (host cells), PVs[associated to papilloma viruses] and [sequences] fragments thereof[, respectively]. However, these attempts have not yet yielded satisfactory results.

BRIEF SUMMARY OF THE INVENTION

[Therefore it is the object of t]The present invention [to]provides a vector having a nucleic acid coding a fusion polypeptide, the fusion polypeptide comprising a structural papillomavirus

(poly)peptide and a non-transforming (poly)peptide coded by an early papillomavirus gene. This fusion polypeptide provides a means[product serving] for activating the immune system to identify and eliminate host cells infected with PVs, PVs and fragments thereof, and in particular[ly] those host cells which have been transformed to a tumorigenic phenotype[cells, associated to papilloma viruses and sequences thereof, respectively]. [According to the invention this is achieved by the subject matters defined in the claims.]

[Thus, the subject matter of the present invention relates to a vector having a nucleic acid coding for a fusion polypeptide, the fusion polypeptide comprising a structural papilloma virus (poly)peptide and a non transforming (poly)peptide coded by an early papilloma virus gene.]

As used herein, [The expression]"vector" comprises any vector which is suitable for gene transfer, [i.e.] for example, those capable of introduc[tion]ing [of] nucleic acids into cells. [The] A vector may remain episomally or be[within the cells or] integrated within the genome of a host cell. Moreover, the vector may be a plasmid or virus vector. The genomes of retroviruses, adenoviruses, vacciniaviruses and adeno-associated viruses (AAV), the latter being preferred, have been adapted as highly efficient vectors for introducing genes into cells.[Examples of a virus vector are retroviral, adenovirus, vacciniavirus or adeno associated virus (AAV) vectors, the latter being preferred.] An AAV vector may be present in wild-type or modified form. In modified form, i[I]t[can also] may comprise [only those sequences such as] two inverted terminal sequences (ITRs) [sequences],that are required[necessary] for its transduction ability. However, depending on the application, it [can]may also be [favorable]advantageous for such a modified AAAV vector to comprise additional sequences,[it to comprise additionally those sequenes,] such as R[r]ep sequences, which [render possible for it the] facilitate the integration of nucleic acids (genes) into chromosome 19. A virus vector can be present as a viral particle or in the form of its nucleic acid. It is preferred for the virus vector to be replication-defective.

[The expression]As used herein, "papillomavirus" comprises any papilloma[ ]virus or [sequences] fragments thereof, which can be found in host[associated with] cells, particularly tumor cells. In particular, HPVs and more particularly "high risk" HPVs, such HPV 16, 18, 33, 35 and 45[, may be concerned].

As used herein,[The expression] "nucleic acid" comprises any nucleic acid such as DNA and/or RNA, which codes for a fusion polypeptide comprising a structural papillomavirus (poly)peptide and a non-transforming (poly)peptide coded by an early papilloma[ ]virus gene. It is favorable for the nucleic acid to be expressible. It is particularly favorable for it to be controlled by a constitutive or inducible promoter such as a tissue-specific or tumor-specific promoter.

As used herein,[The expression] "structural papillomavirus (poly)peptide" comprises any peptide or[and] polypeptide[, respectively] of a papilloma[ ]virus, which is at least [jointly] responsible in part for the structure of the papilloma[ ]virus. In particular, such a (poly)peptide is coded by L1-ORF or L2-ORF, or fragment thereof of a papilloma[ ]virus[and by part thereof, respectively.]. A (poly)peptide which can be present as VLP is particularly preferred.

As used herein, "transformation" refers to the conversion of a normal cell into a tumor cell which has the capacity for unlimited proliferation.

As used herein,[The expression] "a non-transforming (poly)peptide [encoded by an early papilloma virus gene]" comprises any peptide [and]or polypeptide, [respectively, which] that is coded by an early papilloma[ ]virus gene (ORF) or fragment thereof, and is non-transforming by nature or through intervention. The early papillomavirus genes include but are not limited to [particularly the] the E1-, E2-, E4-, E5-, E6- and E7-ORFs. Through intervention, the transforming ability of a (poly)peptide is destroyed by deleting a part of the ORF.[and by part

thereof, respectively, and is non transforming. The expression "non transforming" refers to the fact that the (poly)peptide has no transformation ability by nature or by intervention.] A preferred non-transforming (poly)peptide is coded by a fragment of the E6-[ORF] or E7-ORFs of a papilloma[ ]virus[and by part thereof, respectively].

As used herein,[The expression] "fusion polypeptide" refers to the fact that the structural papilloma[ ]virus (poly)peptide and the non-transforming (poly)peptide coded by an early papilloma[ ]virus gene can be present in any combination with the fusion polypeptide. The individual (poly)peptides may also originate from different papilloma[ ]viruses. The C terminus of the structural (poly) peptides is preferably connected with the N terminus of the non-transforming (poly)peptide. In addition, it may be advantageous for the non-transforming (poly)peptide to be localized within the structural (poly)peptide. A preferred fusion polypeptide comprises a (poly)peptide coded by HPV 16_L1-ORF and a (poly)peptide coded by HPV 16 E6-ORF or[and] E7-ORF, respectively. Furthermore, a fusion polypeptide is preferred which comprises a (poly)peptide coded by HPV 18 L1-ORF and a (poly)peptide coded by HPV 18 E6-ORF or[and] E7-ORF, respectively.

Common methods can be carried out for the preparation of [an] above vectors. For example, an AAV vector can be prepared as a virus particle as follows: [T]the 5' end of the HPV 16 E6-ORF is ligated to the 3' end of the HPV 16 L1-ORF. Part of the E6-ORF has been deleted beforehand, so that the transforming properties of E6 were destroyed. The DNA fragment L1-ORF-E6-ORF is inserted in a[n] modified AAV vector which contains the 5' –ITR and 3' ITR sequences of AAV but not the sequences coding for the AAV Rep and AAV Cap proteins. The insertion is made between the two ITR sequences. The DNA fragment L1-ORF-E6-ORF is controlled by a promoter heterologous with respect to AAV. The resulting AAV vector is transfected into cells,

which express the AAV Rep and AAV-Cap proteins. Furthermore, the cells are infected with a helper virus, e.g. adenovirus, so that the AAV vector is obtained as a viral particle.

The immune system can be activated with the[an] above vector, to identify and eliminate host cells, particularly tumor cells, [associated to]transformed by papilloma[ ]viruses and or PVs and fragments[sequences] thereof, respectively. This can be achieved prophylactically [and in]or as a therapeutic treatment. For this purpose, cells of the particular organism, such as antigen-presenting cells, e.g. dendritic cells, B cells, macrophages and/or tumor cells and/or pre-tumor cells[associated to papilloma viruses and sequences thereof, respectively,] are transduced with the vector. The transduction can be made by common methods. If the vector is available as a virus particle, it will be favorable to infect the cells therewith. On the other hand, if it is available as a nucleic acid, e.g. DNA, it will be advisable to transfect the cells therewith. Electroporation, lipofection and particle gun have to be mentioned as transfection techniques by way of example. The cells may be present in the organism. On the other hand, the cells to be transduced can also be isolated from the organism, [be] transduced outside the organism and then[be] returned to the organism again. Such cells are referred to as autologous cells. Moreover, allogenic cells can also be used for the transduction regarding the organism. In this connection, it is favorable for these cells to belong to an HLA type corresponding to the organism. The person skilled in the art is familiar with processes of providing cells with a certain HLA type. In addition, it is favorable if, in an above process, the tumor cells or pre-tumor cells are inactivated before they are returned to the organism. For this purpose, common methods, such as irradiation, can be used[carried out].

Another subject matter of the present invention relates to a vaccination agent which comprises an above vector and common auxiliary substances, such as buffers, diluents, carriers, etc.. It can be advantageous[favorable] for the vaccination agent to contain additional[further] substances which can activate the immune system, e.g. against tumor cells. Such substances include but are not

limited to[can be particularly] MHC-1 molecules, co-stimulatory molecules, e.g. B7, and secretory immunostimulators, [e.g.]for example, cytokines, such as IL-2, IL-12, interferon and GM-CSF. These substances can be present[e.g.]for example, in the form of peptides, particularly synthetic peptides.

The substances can also be present in the form of expression plasmids encoding them, which can also code for HLA molecules. It is particularly favorable for the vaccination agent to also contain the cells transduced by the vector. Host cells, particularly tumor or pre-tumor cells may be isolated from the organism, transduced by a variety of methods and returned to the organism as a therapeutic treatment as [T]the above explanations apply to the cells as well. If tumor or pre-tumor cells are [concerned]used, it will be favorable for the cells to be inactivated.

By means of the present invention it is possible to activate the immune system against the host cells [which are associated] infected with[to] papilloma[ ]viruses, PVs and or fragments[sequences] thereof, respectively. These cells include[can be] tumor cells and pre-tumor cells, respectively. The activation of the immune system can be made prophylactically or as a therapeutic[and in the] treatment. The present invention represents a new step of treating the most severe diseases via in *in vivo* gene therapy and *ex vivo* gene therapy, respectively.

[The invention is explained by the below example.]

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENT

The present invention is illustrated by the following example relating to the production of viral particles which incorporate the subject fusion polypeptide.

Example: Preparation of a V[v]ector C[c]oding for an HPV16 L1-E7 F[f]usion P[p]olypeptide

The L1-ORF of a genomic HPV16 clone ([cf.]Kirnbauer et al. (1993), J. Virol. 12: 6929-6936) was amplified by PCR reaction. For this purpose, L1-specific primers were used which have an additional Bg1III restriction site at the 5' end. The amplified DNA fragment was cleaved using Bg1III and inserted in BamHI restriction site of the common vector pUC19. An EcoRV restriction site, followed by a translation stop codon (TAA), was introduced at position 7051 of the L1-ORF by specific mutagenesis. By this, it was achieved that the L1-ORF coded for an L1 which was lacking the last 34 amino acids.

In another PCR reaction, the part of the E7-ORF of HPV16 was amplified which codes for the first 50 amino acids of E7. The employed primers included an EcoRV restriction site at their 5' end. The amplified DNA fragment was inserted in the EcoRV restriction site of the above pUC19 vector which codes for the shortened L1. Thus, an L1-E7 fusion gene was obtained. It was inserted in the common baculovirus vector pVL1392 via XbaI/SmaI. The L1-E7 fusion gene was cleaved therefrom by NotI/SmaI and inserted in the NotI restriction site of the AAV vector pUF2 (Zolotukhin et al., J. Virol. 70, (1996), 4646-4654). A vector was obtained which codes for an HPV16 L1-E7 fusion polypeptide. Viral particles of the vector were obtained according to common methods ([cf.]Rolling and Samulski (1995)[,] Molecular Biotechnology 3[,]: [(1995),]9-[ ]15).